

# Hydrogen sulfide and the vasculature: a novel vasculoprotective entity and regulator of nitric oxide bioavailability?

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## Abstract

Hydrogen sulfide (H<sub>2</sub>S) is a well known and pungent toxic gas that has recently been shown to be synthesised in man from the amino acids cystathionine, homocysteine and cysteine by at least two distinct enzymes; cystathionine-γ-lyase and cystathionine-β-synthase. In the past few years, H<sub>2</sub>S has emerged as a novel and increasingly important mediator in the cardiovascular system but delineating the precise physiology and pathophysiology of H<sub>2</sub>S is proving to be complex and difficult to unravel with disparate findings reported with cell types, tissue types and animal species reported. Therefore, in this review we summarize the mechanisms by which H<sub>2</sub>S has been proposed to regulate blood pressure and cardiac function, discuss the mechanistic discrepancies reported in the literature as well as the therapeutic potential of H<sub>2</sub>S. We also examine the methods of H<sub>2</sub>S detection in biological fluids, processes for H<sub>2</sub>S removal and discuss the reported blood levels of H<sub>2</sub>S in man and animal models of cardiovascular pathology. We also highlight the complex interaction of H<sub>2</sub>S with nitric oxide in regulating cardiovascular function in health and disease.

**Keywords:** nitrosothiol • cardioprotection • KATP • vasodilator • gaseous mediators • reactive nitrogen species • sulfur

## Introduction

Hydrogen sulfide (dihydrogen sulfide, H<sub>2</sub>S) is a well known and pungent toxic gas and its toxicology as an environmental pollutant has been extensively studied [1]. Since it was first discovered to be synthesized in human tissues a decade ago, it has attracted substantial interest. Studies in animals have shown H<sub>2</sub>S to be involved in several physiological and pathophysiological processes as diverse as learning and memory, chronic inflammation and the regulation of blood pressure. It will not have escaped the reader's attention that these processes are also tightly regulated by the much better understood gaseous mediator, nitric oxide. The similarity in physiological and pathophysiological effects between nitric oxide and H<sub>2</sub>S has led to speculation about the existence of cellular

'cross-talk' between the two gases and the enzymes responsible for their synthesis.

It should be noted here that although the words sulfur and sulphur are widespread in the scientific literature, predominantly from United States and UK/ UK-Commonwealth based researchers, respectively, an etymological basis for the spelling of sulphur is lacking. Sulfur is Latin and not Greek (the Greek for sulfur being *thion*) although both terms continue to be used interchangeably. However, it should be noted that the International Union of Pure and Applied Chemistry (IUPAC) applied the spelling of 'sulfur' in 1990 [2]. This was subsequently adopted by the nomenclature committee of the Royal Society of Chemistry (UK) in 1992 [3]. As such and to avoid confusion, we refer to H<sub>2</sub>S as hydrogen sulfide.

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## Reactive nitrogen species (RNS) in the heart and vasculature

Nitric oxide and peroxynitrite ( $\text{ONOO}^-$ ) are RNS formed *in vivo* (extensively reviewed in [4]). Nitric oxide is a free radical gas synthesized enzymatically from the amino acid L-arginine in a number of tissues using the three isoforms of nitric oxide synthase (NOS). Classically, NOS1 and NOS-3 are constitutively expressed and require  $\text{Ca}^{2+}$  calmodulin to produce low nM quantities of nitric oxide over short periods of time. NOS2 is an inducible  $\text{Ca}^{2+}$ -independent isoform and synthesizes high  $\mu\text{M}$  concentrations of nitric oxide over a period of hours. Nitric oxide is important in the endothelium-dependent regulation of blood flow and pressure, inhibiting the activation of blood platelets, neurotransmission and memory (long-term potentiation; LTP). Nitric oxide is also involved with host-defence and the inflammatory cascade (exhaustively reviewed in [4]). Since the half-life of nitric oxide at physiological pH is  $<5\text{s}$ , its formation *in vivo* can be quantitated by the measurement of its stable oxidation products, nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ) and from the S-nitrosation of free or protein thiols (S-nitrosothiols) [4].

The roles of nitric oxide and  $\text{ONOO}^-$  in the cardiovascular system have been exhaustively studied and reviewed in great detail elsewhere [4, 7]. Briefly, nitric oxide, through the activation of the  $\text{Ca}^{2+}$ /calmodulin-dependent enzymes NOS1 and NOS3 maintains cardiac function and vascular patency through direct endothelial-dependent interaction with soluble guanylate cyclase resulting in cGMP accumulation, intracellular protein phosphorylation and vasorelaxation. In conditions such as sepsis, shock or inflammation, a third isoform, NOS2 is induced resulting in high concentrations of nitric oxide being synthesized and potentially lethal hypotension. Administration of NOS2 inhibitors effectively attenuate this hypotension in animal models. In otherwise healthy individuals or animals, the administration of NOS3 or more recently NOS1 [8] inhibitors alone can increase blood pressure clearly demonstrating a role for nitric oxide in regulating blood pressure homeostasis under physiological conditions. Similarly, nitric oxide donating drugs exert potent hypotensive actions [9, 10].

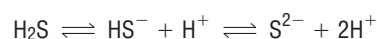
In the vasculature,  $\text{ONOO}^-$  formation through substantially elevated nitric oxide synthesis can lead to endothelial lipid peroxidation, oxidation, nitration and inactivation of  $\text{Ca}^{2+}$ -dependent vascular smooth muscle cell ATPases.  $\text{ONOO}^-$ -mediated oxidation of low-density lipoprotein also leads to the activation of macrophage scavenger receptors to precipitate atherosclerosis as well as directly induce smooth muscle and endothelial cell death. Since this will ultimately lead to impaired vascular response, the physiological removal of  $\text{ONOO}^-$  by antioxidants, decomposition catalysts [4] or thiols (perhaps including hydrogen sulfide [11]) is considered beneficial in maintaining a healthy vasculature.

## H<sub>2</sub>S biosynthesis

H<sub>2</sub>S is rapidly emerging as an important gaseous mediator in the vasculature. In sharp contrast to nitric oxide, and as one would

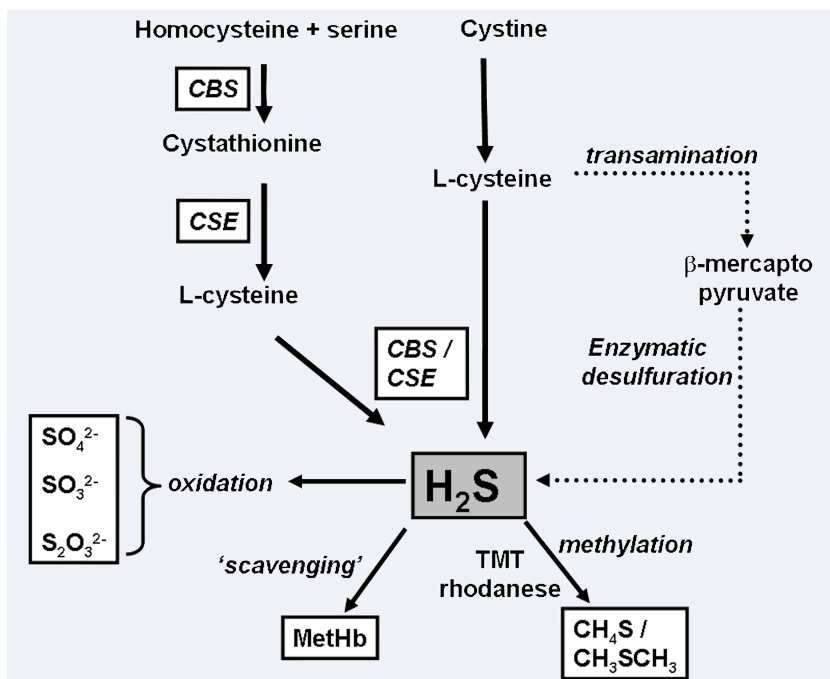
expect from an emerging field of research, its vascular effects, its mechanism of action as well as the processes controlling the regulation of its synthesis are poorly understood. As with nitric oxide, H<sub>2</sub>S is a highly lipophilic molecule and freely penetrates cells of all types. Whether or not H<sub>2</sub>S and nitric oxide exert their effects *in vivo* independently or in tandem is currently not known but a growing body of literature is highly suggestive of H<sub>2</sub>S involvement in the regulation of nitric oxide mediated signalling events and/or *vice versa*.

The bulk of endogenous H<sub>2</sub>S synthesis in mammalian tissues appears to be from the pyridoxal-5'-phosphate-dependent enzymes cystathionine- $\gamma$ -lyase (CSE; E.C. 4.4.1.1) and cystathionine- $\beta$ -synthase (CBS; E.C. 4.2.1.22) and by analogy with NOS use amino acids as substrates; in this case cystathionine, cysteine and homocysteine (summarized in Fig. 1). H<sub>2</sub>S may also be formed *in vivo* from the enzymatic desulfuration of  $\beta$ -mercaptopyruvate derived from cysteine transamination [12] although it is currently uncertain how this pathway contributes to the levels of H<sub>2</sub>S reported in mammalian tissues (see below). It should be noted at this point that in aqueous solution H<sub>2</sub>S is weakly acidic (pK<sub>a</sub> at 37°C, 6.76) and dissociates to form two dissociation states; the hydrosulfide anion ( $\text{HS}^-$ ), pK<sub>a</sub> 7.04 and sulfide anion ( $\text{S}^{2-}$ ), pK<sub>a</sub> 11.96 according to the following sequential reactions

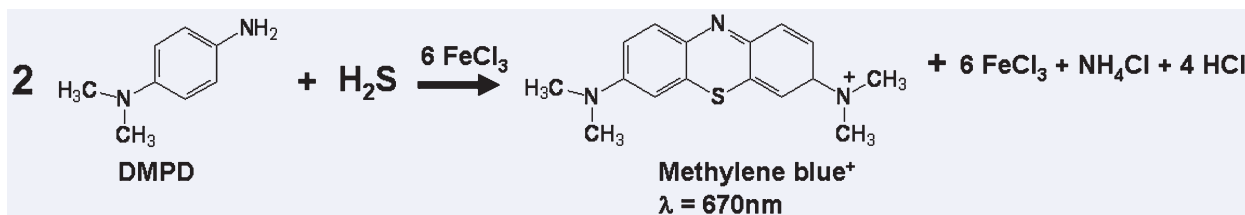


As such, at physiological pH of 7.4 approximately 18.5% of the total sulfide exists as the undissociated acid and 81.5% as the  $\text{HS}^-$  anion [13]. Therefore, since it is currently not known whether the biological effects of H<sub>2</sub>S are mediated directly by H<sub>2</sub>S itself or derived species that will also exist at pH 7.4 (such as  $\text{S}^{2-}$  or  $\text{HS}^-$ ), we prudently use the term H<sub>2</sub>S in this review to reflect the sum of these species present at physiological pH.

The human gene for CSE is located on chromosome 1 (1p31.1) and two possible splice variants of CSE mRNA have been characterized although their precise function in H<sub>2</sub>S synthesis are unknown. CSE expression is induced in liver, kidney and lung by pro-inflammatory mediators such as lipopolysaccharide [14] and also in animal models of type I diabetes mellitus [15] and pancreatitis [16] although the precise molecular pathways for the induced expression of CSE and activity remain elusive. Similarly, the human CBS gene is located on chromosome 21 (21q22.3) [17] and encodes several mRNA [18] but the function of these mRNA isoforms in terms of H<sub>2</sub>S synthesis are not known. However, it has been suggested that the heme component of CBS functions as a cellular redox sensor [19] perhaps increasing H<sub>2</sub>S generation in response to intracellular oxidant load. Interestingly, CBS is allosterically regulated by S-adenosylmethionine [20] and stimulation of CBS activity increases glutathione (GSH) synthesis by promoting cellular cysteine uptake and accompanying increased  $\gamma$ -glutamylcysteine synthetase activity [21, 22] suggesting a role for CBS (and perhaps H<sub>2</sub>S) in addressing intracellular redox imbalances due to increased oxidant load. In agreement, H<sub>2</sub>S or species derived from it at physiological pH, react with several cytotoxic oxidant species such as hypochlorite [23], peroxynitrite [10], hydrogen peroxide,  $\text{O}_2^-$  [24, 25] and nitric oxide [26] and prevent cell death induced by these oxidants suggesting a cytoprotective function for H<sub>2</sub>S.



**Fig. 1** Major Pathways of  $\text{H}_2\text{S}$  synthesis *in vivo*.  $\text{H}_2\text{S}$  is synthesized from the amino acids L-cysteine and L-cystathionine by one of two enzymes (depending on cell type), cystathionine- $\beta$ -synthase (CBS) and cystathionine- $\gamma$ -lyase (CSE). Aminooxyacetate and propargylglycine (PAG) are commonly used inhibitors of CBS and CSE activity, respectively. The most prominent source of  $\text{H}_2\text{S}$  in the vasculature is CSE. Removal of  $\text{H}_2\text{S}$  is thought to occur *via* oxidation to sulfate ( $\text{SO}_4^{2-}$ ), sulfite ( $\text{SO}_3^{2-}$ ) and thiosulfate ( $\text{S}_2\text{O}_3^{2-}$ ), scavenging by methemoglobin (MetHb) or methylation by enzymes such as thiomethyltransferase (TMT) and rhodanese to form methanethiol ( $\text{CH}_4\text{S}$ ) and dimethylsulfide ( $\text{CH}_3\text{SCH}_3$ ).



**Fig. 2** Methylene blue assay for  $\text{H}_2\text{S}$  in biological fluids. This is the most simple and widely used assay for determining  $\text{H}_2\text{S}$  levels in body fluids. Using this and other methods (see Table 1), healthy human blood has been found to contain  $\sim 30\text{--}60\ \mu\text{M}$  of  $\text{H}_2\text{S}$ .  $\text{H}_2\text{S}$  levels are reduced in hypertension and increased during endotoxic or haemorrhagic shock. Modified from [31].

As with the various isoforms of NOS, CSE and CBS are widely expressed in human tissues and cells although a degree of tissue specificity is apparent. CBS is highly expressed in neuronal tissue, in particular in cerebellar Purkinje and hippocampal neurons. In contrast, CSE activity is most notable in blood vessels and peripheral tissues [27]. The liver of several species, including human beings, contain high levels of CSE and CBS expression and activity. Although there has been considerable interest in CSE and CBS over several decades due to their role in the trans-sulfuration pathway and homocysteine metabolism, very little is known about the precise molecular pathways for  $\text{H}_2\text{S}$  synthesis from these enzymes. In neuronal tissue, CBS activity appears  $\text{Ca}^{2+}$ /calmodulin-dependent suggesting that short-term control  $\text{H}_2\text{S}$  synthesis may be achieved by  $\text{Ca}^{2+}$  influx following neuronal depolarization [28] in a similar process to the synthesis of nitric oxide from the calcium/calmodulin-dependent NOS. The high endogenous concentrations of  $\text{H}_2\text{S}$  measured in human, rat and bovine brain (between 50 and  $160\ \mu\text{M}$ ) has led to the suggestion that like nitric oxide,  $\text{H}_2\text{S}$  may function as an endogenous neuromodulator [29, 30].

## $\text{H}_2\text{S}$ measurement, catabolism and removal

At present there are no known specific and reliable stable end products of  $\text{H}_2\text{S}$  biosynthesis in the same way that  $\text{NO}_2^-/\text{NO}_3^-$  function as a robust index of NOS activity *in vivo*. The majority of studies which have evaluated serum or plasma levels of  $\text{H}_2\text{S}$  have used a spectrophotometric approach based around methylene blue. This assay involves the 'fixing' or 'trapping' of aqueous sulfide in biological samples with zinc acetate to prevent unnecessary loss of  $\text{H}_2\text{S}$  through volatilization or through aerial oxidation resulting in the formation of stable zinc sulfide [31]. Upon subsequent acidification,  $\text{H}_2\text{S}$  is released and in the presence of *N,N*-dimethyl-*p*-phenylenediamine (DMPD) and of small quantities of ferric ions, the heterocyclic thiazine dye methylene blue produced can be readily measured using standard laboratory spectrophotometers at 670 nm (Fig. 2) [31] or analysed by high-performance liquid chromatography (see Table 1 for comparison of methods and levels of  $\text{H}_2\text{S}$  reported in mammalian serum and plasma). It is possible that this spectrophotometric assay measures the sum of

**Table 1** Comparison of plasma and serum levels of H<sub>2</sub>S determined by several commonly used techniques: relevance to the cardiovascular system

Species	Model	Method of H <sub>2</sub> S detection	Fluid	H <sub>2</sub> S level reported	Comment	References
Male Sprague-Dawley rats	Myocardial injury induced by homocysteine treatment	Commercial sulfide-sensitive electrode	Plasma	Control rats, ~30 $\mu$ M* Homocysteine treatment, ~50 $\mu$ M*	Homocysteine is a substrate for CSE. H <sub>2</sub> S prevented myocardial injury. CSE expression and activity were lowered by high concentrations of H <sub>2</sub> O <sub>2</sub> (0.1–10mM)	[34]
Rats – species not specified	Isoproterenol-induced myocardial injury	Zinc acetate / <i>N,N</i> -dimethyl- <i>p</i> -phenylenediamine (DMPD)	Plasma	Control rats, ~65 $\mu$ M* Isoproterenol treated rats, ~18 $\mu$ M*	CSE involved in mediating cardiac contraction. Treatment of rats with isoproterenol and NaHS significantly lowered CPK and LDH release from myocardial tissue, lowered the levels of lipid peroxidation products and preserved left ventricular function. NaHS increased survival, induced capillary dilatation and reduced leucocyte infiltration into myocardial tissue. Part of the mechanism for these observations may include H <sub>2</sub> S-mediated 'scavenging' of H <sub>2</sub> O <sub>2</sub> and O <sub>2</sub> <sup>-</sup> .	[25]
Male Sprague-Dawley rats	Endotoxemia induced by bacterial lipopolysaccharide from <i>E. Coli</i>	Zinc acetate / DMPD	Plasma	Control rats, ~26 $\mu$ M* LPS treated rats, ~45 $\mu$ M*	LPS increase plasma PGE <sub>2</sub> levels; an effect decreased by the H <sub>2</sub> S-releasing drug <i>S</i> -diclofenac LPS increased plasma NO <sub>2</sub> <sup>-</sup> levels; decreased by <i>S</i> -diclofenac suggesting H <sub>2</sub> S–nitric oxide cross-talk	[113]
Male Sprague-Dawley rats, WKY rats, Spontaneously hypertensive rats (SHR)	Development of a novel slow releasing H <sub>2</sub> S donor (GYY4137); Hypertension: induced by L-NAME induced hypertension, normotensive rats and SHR	Zinc acetate / DMPD	Plasma	Baseline levels of ~35 $\mu$ M* rose to ~80 $\mu$ M* 90 min after i.v. or i.p. administration of H <sub>2</sub> S donor.	First demonstration of the biphasic effects of H <sub>2</sub> S on cardiac function. Rapid release of H <sub>2</sub> S by NaHS reduced cardiac contractility (left ventricular diastolic pressure) by 42% and heart rate by 53%. In contrast, the slow and sustained release of H <sub>2</sub> S via GYY4137 had no effect on cardiac contractility or heart rate. NaHS increased mean arterial blood pressure in L-NAME treated rats whereas GYY4137 prevented L-NAME-induced hypertension. Furthermore GYY4137 markedly reduced systolic pressure in SHR but not WKY rats.	[79]
Male Sprague-Dawley rats	Type I diabetes induced by streptozotocin (STZ)	Zinc acetate / DMPD	Plasma	Control rats, ~40 $\mu$ M STZ treated rats, ~38 $\mu$ M	Insulin treatment significantly elevated plasma H <sub>2</sub> S levels. STZ elevated liver, kidney and pancreas CSE and CBS expression and activity. STZ is a nitric oxide donating molecule and the decreased plasma levels of H <sub>2</sub> S after STZ treatment could reflect consumption of H <sub>2</sub> S by nitric oxide and / or derived intermediates	[15]

Continued

**Table 1** Continued

Species	Model	Method of H <sub>2</sub> S detection	Fluid	H <sub>2</sub> S level reported	Comment	References
Male Sprague-Dawley rats	High blood-flow induced pulmonary hypertension induced by abdominal aorta-inferior cava vein shunt	Zinc acetate / DMPD	Plasma	Control rats, 50.9 ± 3.9 µM Pulmonary hypertensive rats, 36.4 ± 2.6 µM	Shunt decreased CSE mRNA expression in lung CSE mRNA localized in smooth muscle cells on small pulmonary muscular arteries with minimal expression in endothelial cells	[110]
Male Sprague-Dawley rats	High blood flow induced pulmonary hypertension induced by abdominal aorta-inferior cava vein shunt	Zinc acetate / DMPD	Plasma	Control rats, 50.83 ± 4.01 µM Shunt, 36.42 ± 3.12 µM	Shunting decreased CSE mRNA expression in medial and small pulmonary arteries and rate of H <sub>2</sub> S synthesis in lung tissue	[111]
Male WKY and spontaneous hypertensive (SHR) rats	Hypertension	Zinc acetate / DMPD	Plasma	WKY rats 48 ± 13 µM SHR rats 20 ± 9 µM	PAG treatment decreased H <sub>2</sub> S levels in WKY (to 21 ± 7 µM) and SHR (to 12 ± 10 µM) and also increased the expression of CSE in aorta in WKY and SHR suggesting H <sub>2</sub> S involvement in regulation of blood pressure	[61]
Male Sprague-Dawley rats	Haemorrhagic shock (blood withdrawal, 9-10ml over an hour in 2 min periods)	Zinc acetate / DMPD	Plasma	Prior to blood withdrawal, 28.9 ± 1.4 µM 60 min after blood withdrawal, 37.5 ± 1.3 µM	PAG or β-cyanoalanine injected either prophylactically or therapeutically, inhibited the increase in plasma H <sub>2</sub> S and drop in mean arterial blood pressure. This response was not inhibited by glibenclamide (K <sub>ATP</sub> channel antagonist). Blood withdrawal increased liver CSE mRNA expression	[62]
Wistar rats	Hypoxic pulmonary hypertension (HPH)	Zinc acetate / DMPD	Plasma	Control rats, 301.6 ± 32.41 µM HPH, 92.2 ± 22.1 µM	NaHS increased lung CSE expression; levels of control rats approximately 10 fold higher than the reported findings of others with the same species of rat (see below). Nevertheless, HPH induced a significant decrease in plasma H <sub>2</sub> S.	[43]
Male Wistar rats	Myocardial infarction; ligation of left anterior descending artery from its origin between the pulmonary artery conus and the left atrium	Zinc acetate / DMPD	Plasma	Control rats, 38.2 ± 2.07 µM 48 hrs after MI induction, 59.2 ± 7.16 µM	PAG treatment reduced plasma H <sub>2</sub> S levels to 39.2 ± 5.02 µM whereas treatment with the H <sub>2</sub> S donor, NaHS, significantly increased plasma H <sub>2</sub> S levels to 92.2 ± 12.40 µM. MI and PAG treatment decreased CSE mRNA expression. PAG treatment reduced myocardial oedema and inflammatory cell infiltrate. CSE immunoreactivity detected in infarct area as well as in the endothelium of small vessels of area at-risk. In contrast, CSE was not detected in cardiomyocytes under these experimental conditions.	[70]

Continued

Table 1 Continued

Species	Model	Method of H <sub>2</sub> S detection	Fluid	H <sub>2</sub> S level reported	Comment	References
Male Wistar rats	Hypoxic pulmonary hypertension	Zinc acetate / DMPD	Plasma	Control group, 299.6 ± 12.4 µM Hypoxia, 187.2 ± 13.1 µM	NaHS increased lung CSE expression; levels of control rats approximately 10 fold higher than the reported findings of others with the same species of rat. Administration of NaHS to hypoxia treated rats increased plasma H <sub>2</sub> S levels to 309.2 ± 13.6 µM Potential antioxidant action of H <sub>2</sub> S: Hypoxia decreased plasma antioxidant enzyme superoxide dismutase (SOD), increased levels of oxidized glutathione (GSSG) and lipid peroxidation markers (malondialdehyde); these effects were reversed by NaHS administration.	[114]
Male Sprague-Dawley rats	Cardiac ischemia reperfusion; occlusion of left anterior descending coronary artery	Zinc acetate / DMPD	Plasma	Control group, 58.28 ± 7.86 µM Ischemia-reperfusion group 30.32 ± 5.26 µM	NaHS decreased infarct size and improved haemodynamics (increased left ventricular diastolic pressure) Suggested mechanism for cardioprotection <i>via</i> down-regulation of c-fos expression in myocardium.	[115]
Male Sprague-Dawley rats		Commercial sulfide-sensitive electrode	Plasma	Healthy rats, 45.6 ± 10.59 µM		[109]
Lobund-Wistar (LW) rats Harlan-Sprague-Dawley (HSD) rats C57 Black/6 (C57) mice	Method comparison study	Polarographic H <sub>2</sub> S sensor and Ion-selective electrode Zinc acetate / DMPD	Plasma	H <sub>2</sub> S was not detected in LW or HSD rats or C57 mice. H <sub>2</sub> S was not detected in LW rats but 4.3 ± 0.5 µM detected in HSD rat plasma.	This study also shows the rapid removal, metabolism or sequestration of H <sub>2</sub> S added to vertebrate plasma; half time of decay of 10 µM Na <sub>2</sub> S added to plasma at 37°C, 13.0 ± 0.2 sec.	[38]
Male Swiss albino mice	Septic shock (induced by LPS)	Zinc acetate / DMPD	Plasma	Untreated animals, ~32µM* LPS 4 hrs, ~40µM LPS 20 hrs, ~65µM	LPS induced liver and kidney CSE expression and activity was inhibited by PAG. H <sub>2</sub> S levels correlated with myeloperoxidase expression and activity.	[14]
Male Balb/C mice	Acute pancreatitis induced by caerulein	Zinc acetate / DMPD	Plasma	Control mice, 22.5 ± 1.9 µM Caerulein treatment, 31.1 ± 3.3 µM	Pancreatitis-induced CSE expression elevated plasma H <sub>2</sub> S levels. PAG treatment lowered plasma H <sub>2</sub> S levels, levels pancreatic amylase and myeloperoxidase activity, inhibited acinar cell death and lung injury.	[116]
			Plasma	Control mice, ~30 µM* Caerulein treatment, ~35 µM*		[117]

Continued



Table 1 Continued

Species	Model	Method of H <sub>2</sub> S detection	Fluid	H <sub>2</sub> S level reported	Comment	References
Male Swiss albino mice	Cecal ligation and puncture-induced sepsis (CLP)	Zinc acetate / DMPD	Plasma	Normal, ~10 $\mu$ M* Sham, ~13 $\mu$ M* CLP, 20 $\mu$ M*	Sepsis-induced CSE expression and elevated plasma H <sub>2</sub> S levels. Plasma H <sub>2</sub> S levels were significantly lowered after prophylactic or therapeutic treatment with PAG.	[118]
Female NOR/Ltj and female NOD/Ltj mice	Non-obese diabetic mouse model and controls	Zinc acetate / DMPD	Plasma	Control mice (NOR/Ltj), ~60 $\mu$ M* NOD mice stage I, ~60 $\mu$ M* stage II, ~33 $\mu$ M* stage III, ~27 $\mu$ M*	Plasma levels of H <sub>2</sub> S decreased markedly as disease progressed. L-cysteine stimulated synthesis of H <sub>2</sub> S and vasorelaxant effect in aortic tissue were significantly decreased with increasing disease progression.	[119]
Male C57BL/6J mice	Genetic knock-out studies; Wild-type (CSE <sup>+/+</sup> ), CSE <sup>+/-</sup> and CSE <sup>-/-</sup>	Commercial sulfide ion-selective electrode	Serum	CSE <sup>+/+</sup> mice ~40 $\mu$ M* CSE <sup>+/-</sup> mice ~31 $\mu$ M* CSE <sup>-/-</sup> mice ~18 $\mu$ M*	CSE <sup>-/-</sup> mice had higher systolic blood pressure than CSE <sup>+/-</sup> mice; CSE <sup>+/-</sup> mice had higher systolic blood pressure than CSE <sup>+/+</sup> mice. CSE <sup>-/-</sup> more sensitive to H <sub>2</sub> S-induced vasodilatation than CSE <sup>+/+</sup> .	[55]
Human	Chronic obstructive pulmonary disease (COPD); 27 patients with acute exacerbation of COPD (AECOPD), 37 patients with stable COPD	Commercial sulfide-sensitive electrode	Serum	healthy volunteers; aged 71-80yrs, 35.7 $\pm$ 1.2 $\mu$ M aged 61-70yrs, 34.0 $\pm$ 0.9 $\mu$ M aged 50-60yrs, 36.1 $\pm$ 1.1 $\mu$ M No significant difference between healthy and AECOPD (~35 $\mu$ M*) but elevated to in stable COPD patients (non-smokers, 51.1 $\pm$ 3.0 $\mu$ M; smokers 49.8 $\pm$ 3.8 $\mu$ M)	Smoking significantly lowered plasma levels of H <sub>2</sub> S in healthy controls and AECOPD patients. High levels of nitric oxide (measured as total nitrite/nitrate) correlated to higher H <sub>2</sub> S levels. H <sub>2</sub> S levels also correlated with stage of lung obstruction with COPD* (Stage I, ~72 $\mu$ M; Stage II, ~50 $\mu$ M; Stage III, ~40 $\mu$ M; Stage IV, ~48 $\mu$ M) H <sub>2</sub> S levels negatively correlated with sputum neutrophil count and positively with lung function ( <i>predicted</i> FEV <sub>1</sub> ).	[33]
Human	Chronic obstructive pulmonary disease (COPD); 18 patients before and after theophylline treatment	Commercial sulfide-sensitive electrode	Serum	COPD 30-100 $\mu$ M* Serum levels unaffected by theophylline treatment.	H <sub>2</sub> S levels positively correlated with percentage of predicted FEV <sub>1</sub> , sputum macrophage levels but negatively correlated with sputum neutrophil count. <i>Sputum levels of H<sub>2</sub>S were equivalent to levels of NO<sub>2</sub><sup>-</sup>.</i>	[120]
Human	40 patients with coronary heart disease (CHD), 17 angiographically normal patients	Commercial sulfide-sensitive electrode	Plasma	Normal controls, 51.7 $\pm$ 11.9 $\mu$ M CHD, 26.1 $\pm$ 14.2 $\mu$ M Single vessel CHD, 33.0 $\pm$ 15.0 $\mu$ M Double vessel CHD, 16.9 $\pm$ 7.9 $\mu$ M Multi-vessel CHD, 18.4 $\pm$ 7.8 $\mu$ M Unstable angina, 23.6 $\pm$ 14.4 $\mu$ M Acute myocardial infarction, 19.9 $\pm$ 7.5 $\mu$ M Stable angina, 38.4 $\pm$ 14.5 $\mu$ M CHD with coronary artery occlusion,	Patients with CHD had significantly lower H <sub>2</sub> S levels compared to angiographically normal controls. The number of affected vessels correlated with a decrease in H <sub>2</sub> S levels suggesting decreased H <sub>2</sub> S levels correlate with disease severity but could also reflect either H <sub>2</sub> S consumption by vascular oxidants. Plasma levels were also significantly negatively correlated to blood glucose levels and significantly lower in smokers compared to non-smokers.	[60]

Continued

Table 1 Continued

Species	Model	Method of H <sub>2</sub> S detection	Fluid	H <sub>2</sub> S level reported	Comment	References
Human	Essential hypertension in children	Ionic conductance meter/sulfide specific detector	Plasma	Control group $65.7 \pm 5.5 \mu\text{M}$ (mean age $10.5 \pm 0.73$ ) Hypertensive children (mean age $10.48 \pm 3.2\text{yrs}$ ), $51.9 \pm 6.0 \mu\text{M}$	High plasma levels of H <sub>2</sub> S correlated to low homocysteine concentrations. Hypertensive children had a lower H <sub>2</sub> S: homocysteine ratio compared to the control group ( $5.8 \pm 2.9$ cf. $11.6 \pm 3.3$ ). Higher systolic pressure was associated with a lower plasma H <sub>2</sub> S:homocysteine ratio	[95]
Human	Healthy controls	Gas chromatography-mass spectrometry with pentafluorobenzyl bromide dramatization	Whole frozen blood	35–80 $\mu\text{M}$	Method evaluation paper.	[35]
Human	Healthy volunteers	Microdistillation and ion chromatography	Plasma	Levels differed with varying dietary meat intake Meat free, $42 \pm 15 \mu\text{M}$ 240 g meat, $47 \pm 12 \mu\text{M}$ 420 g meat, $25 \pm 0.8 \mu\text{M}$	This study also directly compared the methylene blue assay with this chromatographic technique and found the levels obtained in both systems to be comparable	[36]
Human	Healthy volunteers and septic shock patients	Zinc acetate / DMPD	Plasma	Age and sex matched healthy controls, $43.8 \pm 5.1 \mu\text{M}$ Septic shock, $150.5 \pm 43.7 \mu\text{M}$		[14]

\*Estimated by the authors from published figures.

H<sub>2</sub>S-derived species such as HS<sup>−</sup> and S<sup>2−</sup> that exist at physiological pH rather than H<sub>2</sub>S itself.

However, the majority of the reported studies been useful in generating a substantial amount of data for researchers to 'benchmark' their results and clearly highlight the emerging importance of H<sub>2</sub>S and closely related species in the cardiovascular system. Levels of H<sub>2</sub>S in the plasma measured using this spectrophotometric assay are generally comparable to other approaches (see Table 1). For example, Chen *et al.* [33] utilized a sulfide-selective electrode and detected serum H<sub>2</sub>S levels in man of  $37.9 \pm 0.9 \mu\text{M}$  (healthy controls) cf.  $33.0 \pm 0.7 \mu\text{M}$  (smokers) and  $51.1 \pm 3.0 \mu\text{M}$  (chronic obstructive pulmonary disease [COPD] patients). Using a similar electrochemical method Chang *et al.* [34] reported plasma H<sub>2</sub>S levels in rat of 30–50  $\mu\text{M}$ . Furthermore, the analysis of human plasma by more laborious analytical techniques such as gas chromatography-mass spectrometry with pentafluorobenzyl bromide dramatization or microdistillation with ion chromatography revealed H<sub>2</sub>S levels of 35–80  $\mu\text{M}$  [35] and 25–50  $\mu\text{M}$  [36],

respectively. We recently reported comparable serum levels of H<sub>2</sub>S in healthy human volunteers using the methylene blue spectrophotometric assay and found mean serum levels ( $\pm$ S.D.) of  $43.8 \pm 5 \mu\text{M}$  which were increased markedly (up to 200  $\mu\text{M}$  in one case) during septic shock [14]. As such, the methylene blue based spectrophotometric assay appears to be convenient, relatively robust and at least comparable to other techniques for the measurement of plasma H<sub>2</sub>S ('free' H<sub>2</sub>S, HS<sup>−</sup> or S<sub>2</sub><sup>−</sup>).

However, it is prudent to note that while the majority of laboratories report plasma H<sub>2</sub>S in the region of 25–80  $\mu\text{M}$  it is important to note here that a few laboratories have reported markedly different findings using the same analytical techniques. For example, Chenyu *et al.* [37] have reported levels of in excess of 300  $\mu\text{M}$  in plasma from healthy rats whereas others [38, 39] have suggested that the actual level of H<sub>2</sub>S in plasma was beyond the limit of detection for the methylene blue assay and electrochemical detectors (Table 1). Indeed, using rat tissue homogenates and head space gas chromatography Furne *et al.* [39] failed to detect tissue



release of H<sub>2</sub>S from the tissues into the head space after supplementing tissues with L-cysteine. These important studies do not necessarily contradict the majority of the reported findings in the literature (see Table 1) as they would suggest that H<sub>2</sub>S is sequestered or carried in plasma and tissues rather than released as 'free' H<sub>2</sub>S. Indeed, it is intriguing to note here that when solutions of H<sub>2</sub>S or the commonly used sulfide salt donors (Na<sub>2</sub>S and NaHS) are prepared in the laboratory at concentrations reported in plasma, the solutions are characteristically pungent. However, blood, plasma, serum or tissue homogenates, while containing H<sub>2</sub>S (Table 1) do not smell, further suggesting that the widely used assays could measure a form of bound or sequestered H<sub>2</sub>S rather than 'free' H<sub>2</sub>S.

Whatever the absolute level of H<sub>2</sub>S in plasma, it is likely that any reported plasma levels could represent an underestimate of the true extent of H<sub>2</sub>S synthesis as pathways for H<sub>2</sub>S removal exist (summarized in Fig. 1) although it is uncertain how rapid this process occurs in the vasculature. The half-life of H<sub>2</sub>S in mammalian plasma has been estimated at <30 min. although the mechanism for this has not been identified [38]. A direct reaction with methemoglobin forming sulfhemoglobin has been suggested and it is possible that sulfhemoglobin could act as a 'sink' for circulating H<sub>2</sub>S as it releases H<sub>2</sub>S upon reduction. Intracellular pathways for H<sub>2</sub>S removal also exist. In cells, H<sub>2</sub>S is rapidly oxidized to thiosulfate (S<sub>2</sub>O<sub>3</sub><sup>2-</sup>) by mitochondria and subsequently converted to sulfite (SO<sub>3</sub><sup>2-</sup>) and the major and stable product, sulfate (SO<sub>4</sub><sup>2-</sup>) [40, 41]. Unfortunately, SO<sub>4</sub><sup>2-</sup> cannot be used as a 'bio-marker' of endogenous H<sub>2</sub>S production since it can be derived from the direct oxidation of cysteine by cysteine dioxygenase (E.C. 1.13.11.20) and oxidation of SO<sub>3</sub><sup>2-</sup> by sulfite oxidase (E.C. 1.8.3.1) [42].

Urinary and blood S<sub>2</sub>O<sub>3</sub><sup>2-</sup> has been used as a marker of environmental H<sub>2</sub>S inhalation [43, 44] and elevated urinary S<sub>2</sub>O<sub>3</sub><sup>2-</sup> has been measured in Down syndrome patients [45] with the suggestion that these reflected an overproduction of H<sub>2</sub>S in Down syndrome patients although blood S<sub>2</sub>O<sub>3</sub><sup>2-</sup> levels were not determined in this study. However, S<sub>2</sub>O<sub>3</sub><sup>2-</sup> may not be completely reliable as an index of H<sub>2</sub>S since S<sub>2</sub>O<sub>3</sub><sup>2-</sup> levels in blood without detection in urine have also been observed after industrial H<sub>2</sub>S poisoning [46, 47] and urinary S<sub>2</sub>O<sub>3</sub><sup>2-</sup> in otherwise healthy individuals vary greatly and are sensitive to small fluctuations in atmospheric H<sub>2</sub>S [47]. In animal studies where SO<sub>3</sub><sup>2-</sup>, SO<sub>4</sub><sup>2-</sup> and S<sub>2</sub>O<sub>3</sub><sup>2-</sup> have been measured simultaneously, H<sub>2</sub>S exposure resulted in a greater elevation of SO<sub>3</sub><sup>2-</sup> and SO<sub>4</sub><sup>2-</sup> compared to S<sub>2</sub>O<sub>3</sub><sup>2-</sup> in lung tissue [48]. Similarly, incubation of plasma, liver, colonic or muscle tissue with H<sub>2</sub>S or one of its metabolites methanethiol (see below) also leads to S<sub>2</sub>O<sub>3</sub><sup>2-</sup> and SO<sub>4</sub><sup>2-</sup> generation [49]. These findings strongly suggest that the metabolism of H<sub>2</sub>S *in vivo* is complex and the reliability of S<sub>2</sub>O<sub>3</sub><sup>2-</sup> as an index of endogenous H<sub>2</sub>S synthesis requires further attention.

Further cellular H<sub>2</sub>S removal processes also exist. For example, in the cytosol, H<sub>2</sub>S also undergoes methylation in by thiol-S-methyltransferase (E.C. 2.1.1.9) to yield methanethiol (CH<sub>3</sub>S) and dimethylsulfide (CH<sub>3</sub>SCH<sub>3</sub>) [49, 50]. An additional enzymatic removal process which is potentially important in colonic tissue

involves rhodanese (thiosulfate : cyanide sulfurtransferase; E.C. 2.8.1.1) [51], an enzyme which catalyses the transfer of the sulfane sulfur atom from HS<sup>-</sup> to a thiophilic acceptor (such as cyanide) *via* an enzymic persulfide intermediate to form thiocyanate (SCN<sup>-</sup>) and SO<sub>4</sub><sup>2-</sup>. H<sub>2</sub>S can also be oxidized by activated neutrophils to SO<sub>3</sub><sup>2-</sup> [52] suggesting H<sub>2</sub>S may represent an important source of SO<sub>3</sub><sup>2-</sup> *in vivo*. Furthermore, H<sub>2</sub>S could also be consumed by endogenous oxidant species in the vasculature such as peroxynitrite [10], hypochlorite [23], superoxide [24, 25], hydrogen peroxide [24, 25] and nitric oxide [26], mediators also known to be produced in elevated amounts in various cardiovascular pathologies.

## H<sub>2</sub>S in the heart and vasculature

H<sub>2</sub>S is emerging as a novel and an important physiological mediator in the cardiovascular system. Much of our current knowledge of the cardiovascular biology of H<sub>2</sub>S stems from the use of inhibitors of CSE such as D,L-propargylglycine (PAG), β-cyanoalanine and inhibitors of CBS such as aminooxyacetic acid. These compounds apparently target the pyridoxal phosphate binding site of these enzymes [53] and may not be entirely specific. It should also be noted that although intraperitoneal injection of PAG results in its rapid accumulation into the blood (peak within 2 hrs), an as yet unidentified metabolite of PAG produced through the D-amino acid oxidase pathway injures the renal proximal tubule cells resulting in significant polyuria, proteinuria and glucosuria 5–6 hrs after injection [54]. As such, a possible diuretic and/or other renal effects of this PAG metabolite (or PAG itself) in mediating the effects of PAG (and H<sub>2</sub>S) on systemic blood pressure should be considered.

Nevertheless, in the present absence of wholly specific inhibitors in this emerging field of research, studies using PAG when combined with analytical techniques such as PCR, immunohistochemistry and Western blotting or more recently using animal knock-out models [55] have clearly shown CSE to be the more important of the two known H<sub>2</sub>S synthesizing enzymes in the cardiovascular system [55, 56], where its expression is induced by pro-inflammatory mediators such as lipopolysaccharide and carrageenan but down-regulated during hypoxia, hypertension and myocardial infarction (see Table 1). In sharp contrast, very little is known about the role of H<sub>2</sub>S, CSE or CBS in human beings. To date only, one study has shown CSE to be present in human vascular tissue (internal mammary artery) [57] although it is known to be expressed in human liver [58] and leucocytes [59]. Nevertheless, the few emerging studies examining H<sub>2</sub>S levels in human blood have clearly shown perturbed H<sub>2</sub>S synthesis in coronary heart disease [60] and myocardial infarction, where lower H<sub>2</sub>S levels have been suggested to reflect disease severity (see Table 1).

Although experiments in man and animals have clearly shown that removal of endogenous nitric oxide through inhibition of NOS (*i.e.* with L-NAME) rapidly increases blood pressure, contrasting effects of CSE inhibition (*i.e.* with PAG) have been reported. For example, injection of PAG exerted little immediate effect on blood

pressure in normotensive anaesthetized rats [61, 62] but increased blood pressure after endotoxic [14] or haemorrhagic shock [52] when administered either prophylactically or therapeutically. This suggested CSE-derived H<sub>2</sub>S as a pathological mediator in blood pressure homeostasis. In sharp contrast, longer-term injection of PAG in rats for 2–3 weeks significantly increased systolic blood pressure [63] suggesting the role of CSE was regulatory, rather than pathological. These disparities could either reflect the manner in which PAG was administered or merely the extent to which PAG is cell permeable in different animal models systems and different animal strains. Although PAG reduces H<sub>2</sub>S levels in the plasma of various animal models (see Table 1) and the activity of CSE in isolated cells *in vitro*, it has been so far assumed that PAG is freely cell permeable. However, to the best of our knowledge, studies explicitly showing cellular uptake of PAG are lacking. Nevertheless, by far the most compelling evidence for the role of H<sub>2</sub>S in the vasculature is from very recent studies using CSE single and double knock-out mice [55] where CSE<sup>-/-</sup> and lesser extent CSE<sup>+/-</sup> mice had significantly higher systolic arterial blood pressure than CSE<sup>+/+</sup> mice. Furthermore, CSE<sup>-/-</sup> mice were substantially more sensitive to H<sub>2</sub>S-mediated vasodilatation than wild-type mice [55].

Plasma or serum levels of H<sub>2</sub>S in rat, mouse and human adult plasma vary between ~23 and 60  $\mu$ M (summarized in Table 1). Within these reported plasma concentration ranges, H<sub>2</sub>S induces the relaxation and transient blood pressure reduction in a variety of vascular tissue such as rat aorta and portal vein [64, 65], rabbit corpus cavernosum [66] as well as perfused rat mesenteric [67] and hepatic [68] vascular beds. H<sub>2</sub>S has been shown to be produced in myocardial tissue by CSE *in vitro* and *in vivo* to protect against hypoxia-reperfusion injury, where it exerts a negative inotropic effect and preserves left ventricular function by lowering left ventricular systolic and end diastolic pressure and reducing infarct size [25, 69–72]. The mechanism for the vasculoprotective effects H<sub>2</sub>S (summarized in Table 2) may be mediated by a direct stimulation of plasma membrane K<sub>ATP</sub> channel-dependent vasorelaxation resulting in increased vascular flow. Rodent aortic ring preparations exposed to high concentrations of K<sup>+</sup> ions show markedly reduced vasorelaxant response to H<sub>2</sub>S and K<sub>ATP</sub> channel antagonists such as glibenclamide or 5-hydroxydecanoate (5HD) effectively blocked H<sub>2</sub>S-mediated vasodilatation [65, 70, 71]. Patch clamp studies using isolated rat mesenteric [73] and aortic [74] vascular smooth muscle cells exposed to H<sub>2</sub>S show increased glibenclamide-inhibitable K<sub>ATP</sub>-dependent current and cellular hyperpolarization [75]. However, H<sub>2</sub>S-mediated opening of K<sub>ATP</sub> channels does not occur in isolated rat cardiomyocytes [76] suggesting the effects of H<sub>2</sub>S on K<sub>ATP</sub> may be cell specific.

Additional pathways for H<sub>2</sub>S-mediated vasodilation have also been proposed. For example, H<sub>2</sub>S activates adenylate cyclase and elevates cAMP in cultured neurons [30, 32] and isolated human vascular smooth muscle (HVSM) cells [77], suggesting a vasodilatory effect mediated through cAMP is also possible. On the other hand, the activation of this cAMP pathway was not observed in isolated rat cardiomyocytes where the inhibition of L-type Ca<sup>2+</sup> channels was proposed [76] or rat vascular endothelial

cells [78]. These findings further suggest cell and perhaps species specific effects of H<sub>2</sub>S on vascular cells as have been observed with nitric oxide.

H<sub>2</sub>S is further reported to stimulate charybdotoxin/apamin-sensitive K<sup>+</sup> channels in vascular endothelium [73, 75] although other laboratories have not observed this [79]. Nevertheless, it is highly likely that, as with nitric oxide the vascular endothelium represents the major target for H<sub>2</sub>S [55, 73, 75, 79]. Consistent with this, reduced CSE expression/activity and decreased H<sub>2</sub>S concentration contributes to the pathophysiology of pulmonary hypertension in rodents [80] whereas CBS deficiency leads to hyperhomocyst(e)inemia, increased blood pressure and endothelial dysfunction [81, 82]. Although CBS is not considered to be the vascular source of H<sub>2</sub>S [55], tissue deficiencies in CBS are thought to manifest in the vasculature as a consequence of homocysteine accumulation (reviewed in [83, 84]). Homocysteine is directly toxic to vascular endothelial cells (reviewed in [85, 86]) and induces apoptotic cell death [87], up-regulates surface expression of intracellular adhesion molecule (ICAM)-1 [88], augments monocyte adhesion [89] and promotes interleukin (IL)-1 $\beta$  and tumour necrosis factor- $\alpha$  formation [90] and interferes with vascular nitric oxide synthesis and bioavailability [91–94]. Interestingly, homocysteine treatment of Sprague-Dawley rats markedly increased plasma H<sub>2</sub>S levels *via* increased expression and activity of CSE [34] and it is tempting to speculate that this response by vascular cells to synthesize vasodilatory H<sub>2</sub>S in the presence of a detrimental vascular toxin was a protective and/or compensatory response to maintain vascular patency.

In rodents, CBS or CSE deficiency induced by genetic deletion or chronic treatment with PAG results in a severe hypertension and severe loss of endothelial function [55, 82]. Interestingly, a close examination of the published literature clearly shows that spontaneously hypertensive rats have lowered H<sub>2</sub>S concentrations than rats from other species (*i.e.* WKY, Wistar, Sprague-Dawley) (Table 1). Furthermore, rodent models of pulmonary and hypoxic hypertension also show decreased plasma H<sub>2</sub>S levels. In each case, the administration of the H<sub>2</sub>S donor, NaHS, reduced blood pressure. Interestingly, children are reported to have higher plasma levels of H<sub>2</sub>S than adults [95] and one study has shown that plasma H<sub>2</sub>S concentrations are decreased with age in adults over 50–80 years old [33]. Together with the observation that H<sub>2</sub>S-mediated vasodilation is at least partly endothelium dependent [65, 73–75, 79]; this suggests that there is a possible loss of H<sub>2</sub>S synthesizing capacity or bio-availability over time that parallels age-associated increases in blood pressure in man.

It is possible that part of the vasculoprotective effects may be due to inhibition of platelet aggregation [96]. However, this is unlikely since significant inhibition platelet aggregation by H<sub>2</sub>S (~30  $\mu$ M) at physiologically relevant concentrations was only observed with ADP and millimolar concentrations of NaHS were required to inhibit platelet aggregation induced by other mediators such as collagen, arachidonate and thrombin. It is further likely that H<sub>2</sub>S removes toxic oxidants that are known to be detrimental to endothelial function in the vasculature [11, 23–26]. An additional protective effect, consistent with a potential antioxidant role of H<sub>2</sub>S, may be in limiting vascular protein modification such as

**Table 2** Summary of effects consistent with a protective role for H<sub>2</sub>S in the vasculature

Observation	Example	Comment	References
Antioxidant activity	Scavenging or removal of toxic radical and radical-derived intermediates (i.e. ONOO <sup>-</sup> , HOCl, O <sub>2</sub> <sup>-</sup> , H <sub>2</sub> O <sub>2</sub> , nitric oxide)	Cytoprotective effects observed in neuronal cells but potent <i>in vitro</i> oxidant scavenging also observed so it is possible the interaction with H <sub>2</sub> S and these oxidants also occur in the vasculature. Indeed, H <sub>2</sub> O <sub>2</sub> / O <sub>2</sub> <sup>-</sup> 'scavenging' prevented myocardial injury <i>in vitro</i> .	[11, 23–26]
		3-nitrotyrosine (a bio-marker for ONOO <sup>-</sup> ) and MDA levels (a bio-marker for lipid peroxidation) in rat heart were reduced in a model of myocardial ischemia reperfusion	[99]
	Inhibition of oxidative modification to low-density lipoprotein	Oxidation of low-density lipoprotein considered pro-atherogenic	[97]
	Inhibition of NADPH oxidase synthesis and O <sub>2</sub> <sup>-</sup> formation in human vascular smooth muscle cells	Down-regulation of NADPH oxidase expression. Possible involvement of cAMP and protein kinase A	[77]
	NaHS induces glutathione (GSH) synthesis and decreases plasma levels of oxidized glutathione (GSSG).	Effects observed in neuronal cells. Increased cysteine uptake and increased activity of $\gamma$ -glutamylcysteine synthetase; GSH is an effective extracellular and intracellular antioxidant NaHS increased plasma total antioxidant capacity	[21, 22, 30, 114]
Effects on vascular cells	Inhibition of vascular smooth muscle cell proliferation	Activation ERK and p21 <sup>cip/WAF</sup> -mediated pathway; observed in rat and human cells either treated with NaHS or through over-expression of CSE.	[100, 102]
		However, inhibition of ERK has also been reported.	[101]
	Induction vascular smooth muscle cell apoptosis	Human artery smooth muscle cells. ERK and p38 MAPK activation, up-regulation of p21 <sup>cip/wak-1</sup> and down-regulation of cyclin D1 expression. Caspase-mediated cell death.	[103, 104]
	Induction of endothelial cell adhesion, migration and proliferation	AKT and PI-3K-dependent pathways	[98–102]
	Inhibition of myocardial apoptosis	Rat model of myocardial ischemia-reperfusion injury. NaHS-mediated inhibition of apoptosis was abolished by the putative mitochondrial K <sub>ATP</sub> channel inhibitor 2-HD. JNK, p38 and NF- $\kappa$ B-dependent pathways also activated.	[99]
	Promotion of angiogenesis	AKT pathway proposed.  NaHS inhibited adhesion molecule expression (ICAM-1) in rat model of myocardial ischemia reperfusion	[78] [99]

Continued

Table 2 Continued

Observation	Example	Comment	References
<b>H<sub>2</sub>S required for cardiovascular health</b>			
Vasodilator effects	Vasodilation <i>via</i> opening K <sub>ATP</sub> channel in smooth muscle and vascular endothelium; Induces relaxation and transient blood pressure reduction in rat aorta, portal vein and mesenteric and hepatic vascular beds; induces relaxation of rabbit corpus cavernosum; induces relaxation in human internal mammary artery.	H <sub>2</sub> S-mediated vasodilation was endothelium dependent and inhibitable by glibenclamide or 5HD suggesting K <sub>ATP</sub> -channel-dependent effects	[57, 64–68, 74–76, 79]
	Inhibition of angiotensin-converting enzyme activity of endothelial cells	If this reaction occurred <i>in vivo</i> it could potentially lead to vasodilation <i>in vivo</i> since inhibition of angiotensin-converting enzyme would decrease plasma levels of the angiotensin-II and decrease the breakdown of bradykinin.	[105]
		Presumably the effect of H <sub>2</sub> S on angiotensin-converting enzyme was due to H <sub>2</sub> S-interaction with the active site Zn <sup>2+</sup> ions since exogenous Zn <sup>2+</sup> prevented enzyme inhibition. However, the effect could also be due to direct 'scavenging' effect of Zn <sup>2+</sup> ions on H <sub>2</sub> S as it is the basis of the methylene blue assay	
	Activation of adenylyl cyclase and cAMP-mediated vasodilation	NaHS increased cAMP in human vascular smooth muscle cells;	[77]
		NaHS also induces cAMP formation in cultured neurons and macrophages suggesting one additional mechanism for H <sub>2</sub> S-mediated vasodilation is mediated through adenylyl cyclase / cAMP.	[30, 32]
		However this effect was not observed in isolated rat cardiomyocytes or rat vascular endothelial cells highlighting potential cell-specific and species-dependent effects of H <sub>2</sub> S.	[75, 77]
Low plasma levels implicated in cardiovascular disease	Spontaneously hypertensive rats have lower plasma H <sub>2</sub> S levels than normotensive WKY, Wistar or Sprague-Dawley rats	Data consistent with H <sub>2</sub> S as a physiological regulator of blood pressure	See Table 1 for comparison of levels between different rat strains
	Low CSE expression, activity and H <sub>2</sub> S levels contribute to the pathology of hypertension, coronary heart disease, angina, hypoxia and ischemia-reperfusion injury	Data consistent with H <sub>2</sub> S as a physiological regulator of blood pressure and cardiac function; low plasma H <sub>2</sub> S reflected severity of cardiovascular disease.	[15, 25, 33, 60, 61, 79, 95, 110, 113, 114] [55]
		Low levels could suggest decreased H <sub>2</sub> S synthesis or consumption by toxic intermediates such as nitric oxide, ONOO <sup>-</sup> , O <sub>2</sub> <sup>-</sup> HOCl or H <sub>2</sub> O <sub>2</sub> .	
	CSE <sup>-/-</sup> mice have higher blood pressure than CSE <sup>+/-</sup> and CSE <sup>+/+</sup> mice		

Continued

Table 2 Continued

Observation	Example	Comment	References
	Slow release H <sub>2</sub> S donor decrease systolic blood pressure in spontaneously hypertensive rats but not normotensive WKY rats	Effects of NaHS compared with a novel slow releasing H <sub>2</sub> S donor; biphasic effects of H <sub>2</sub> S	[79]
	Plasma H <sub>2</sub> S levels decline with increasing age.	H <sub>2</sub> S-mediated vasodilation is endothelium dependent. Age-dependent increase in blood pressure and endothelial dysfunction are well documented. Data consistent with H <sub>2</sub> S as a physiological regulator of blood pressure.	[33]
		Interestingly, hypertensive children had lower H <sub>2</sub> S than normotensive children. Normotensive children have higher plasma H <sub>2</sub> S than adults.	[95]
<b>Cardiovascular protective effects of H<sub>2</sub>S</b>			
Protection against myocardial ischemia-reperfusion and myocardial infarction injury	Preserves left ventricular contractility and reduces infarct size	Opening of mitochondrial K <sub>ATP</sub> channels and up-regulation of Bcl <sub>2</sub> protein to prevent mitochondrial permeability and release of pro-death factors ( <i>i.e.</i> cytochrome <i>c</i> ) <i>i.e.</i> H <sub>2</sub> S is cytoprotective. Inhibition of c-Fos signalling. Inhibition of pro-inflammatory signalling (p38, JNK and NF- $\kappa$ B)	[69–72, 99, 115]
	Preservation of mitochondrial ultrastructure and respiratory function during ischemia reperfusion		
	H <sub>2</sub> S is a mediator in ischemic preconditioning	H <sub>2</sub> S activated AKT, PKC- $\alpha$ and PKC $\xi$ -dependent signalling	[121]
	Inhibition of platelet aggregation	Concentrations of H <sub>2</sub> S within the physiological range (~30 $\mu$ M) prevented ADP-induced platelet aggregation. However, mM concentrations of H <sub>2</sub> S required to inhibit platelet aggregation <i>via</i> other mediators. Plasma H <sub>2</sub> S unlikely to reach mM concentrations.	[96]
	Inhibition of L-type Ca <sup>2+</sup> channels in isolated rat cardiomyocytes	NaHS inhibited electricity-stimulated intracellular Ca <sup>2+</sup> mobilization in a K <sub>ATP</sub> -channel independent manner. Potential to reduced myocardial hypertrophy and inhibit cardiomyocyte apoptosis.	[76]

inhibiting HOCl-mediated atherogenic modification of low-density lipoprotein and apolipoprotein and inhibit myeloperoxidase activity, presumably by interaction at the heme moiety of myeloperoxidase [97].

Alternatively, the cytoprotective effects of H<sub>2</sub>S may be due to the initiation of 'protective cellular' signalling pathways within cardiac and vascular tissues. For example, H<sub>2</sub>S induces extracellular regulated kinase (ERK) and protein kinase B (AKT) signalling path-

ways in cardiac tissue [78, 98, 99] and preserves mitochondrial ultrastructure and respiratory chain function *in vivo* [72] possibly *via* a mitochondrial pathway involving the preservation of Bcl-2 signalling and intriguingly, a mitochondrial K<sub>ATP</sub>-channel-dependent mechanism [99]. H<sub>2</sub>S is also reported to induce vascular angiogenesis and induced neovascularization in mice *in vivo* as well as promote proliferation, adhesion and migration in cultured endothelial cells *via* AKT and phosphatidylinositol 3-kinase phosphorylation



[78, 98]. Furthermore, H<sub>2</sub>S was recently shown to inhibit neointima formation after balloon injury in rat carotid artery *in vivo* by inhibiting vascular smooth muscle cell proliferation [100, 101], an effect also observed with HVSM cell proliferation *in vitro* by either inducing or inhibiting [101] ERK and p21<sup>cip/WAF</sup> phosphorylation [102]. Furthermore a pro-apoptotic effect of H<sub>2</sub>S on HVSM cells has also been proposed, also mediated through ERK/p21<sup>cip/wak-1</sup> [103] and p38 pathways involving caspase-3 activation [103, 104] suggesting that the role of H<sub>2</sub>S in the vasculature is to activate the endothelium and inhibit vascular smooth muscle cell function. Indeed, as with nitric oxide mediated vasorelaxation, the vasodilatory effects of H<sub>2</sub>S are endothelium dependent [65, 73–75, 79].

An additional effect of H<sub>2</sub>S may be due to the inhibition of angiotensin-converting enzyme on the surface of vascular endothelial cells [105] possibly resulting from an interaction of H<sub>2</sub>S with the Zn<sup>2+</sup> moiety at the enzyme's active site. Should the corresponding reaction occur *in vivo* this would result in vasodilation due to lowered circulating levels of the vasoconstrictor angiotensin-II and consequently decrease the breakdown of the vasodilator bradykinin. Since it is also likely that H<sub>2</sub>S interacts with NOS3 and endothelial release of nitric oxide, this suggests that these gases may be reciprocally regulated (see below). Indeed, H<sub>2</sub>S appears to activate adenylate cyclase/cAMP [30, 32, 77] signalling in certain cell types (but not others [76, 78]) whereas nitric oxide mediated effects are *via* guanylate cyclase/cGMP perhaps suggestive of complementary or competing pathways for the control of vascular tone.

Although the bulk of these publications to date have suggested that H<sub>2</sub>S is an endogenous vasodilator, concentrations of H<sub>2</sub>S towards the lower end of that measured physiologically have also shown that H<sub>2</sub>S induces endothelium-dependent vasoconstriction in rat [65] and human arteries [57]. In non-mammalian vertebrates, H<sub>2</sub>S induces vasorelaxation or vasoconstriction (or both) [13, 106]. In isolated bovine pulmonary arteries H<sub>2</sub>S causes tissue contraction whereas it induces a complex contraction–relaxation–contraction response in rat pulmonary arteries [106]. Indeed closer examination of the pioneering publications which examined the vasodilator effects of H<sub>2</sub>S on isolated rat aortic rings clearly show that low concentrations of H<sub>2</sub>S induce contraction [74, 75, 80]. Although this was not stated in the text of these publications, these findings are clearly visible in the published figures. We recently expanded these early studies and showed that in phenylephrine pre-contracted rat aortic ring preparations [65] and isolated human internal mammary artery [57], low concentrations of H<sub>2</sub>S-induced tissue contraction followed by relaxation. Both of these effects were dependent on intact endothelial cells since denuded tissue showed neither tissue contraction (at low H<sub>2</sub>S concentrations) or relaxation (at higher H<sub>2</sub>S concentrations) [65]. Furthermore we also showed that low concentrations of H<sub>2</sub>S-induced endothelium-dependent vasoconstriction *in vitro* and in anaesthetized rats this was inhibited by removal of endogenous nitric oxide with L-NAME [65] again suggesting molecular 'cross-talk' between H<sub>2</sub>S and nitric oxide.

## Evidence for 'cross-talk' between nitric oxide and H<sub>2</sub>S

H<sub>2</sub>S-induced vasorelaxation is only partially blocked by glibenclamide when used at a concentration of 10  $\mu$ M [57, 65, 73–75, 79], which is an appropriate concentration for K<sub>ATP</sub> specificity. This implies the involvement of additional K<sub>ATP</sub>-dependent mechanisms for these vascular effects. Together with the studies highlighted above, these findings strongly suggest the possibility of 'cross-talk' between vascular nitric oxide and H<sub>2</sub>S. However, the precise nature of such an interaction has proved difficult to characterize accurately. Recent studies in vascular tissue and cells from several species have shown H<sub>2</sub>S either acts at the level of NOS, nitric oxide influences CSE activity and that H<sub>2</sub>S (or species derived from it at physiological pH) interact with vascular-derived nitric oxide and *vice versa* with apparent formation of a 'nitrosothiol'-like species. A summary of the present evidence consistent with cross-talk between H<sub>2</sub>S and nitric oxide is presented in Table 3.

The direct effects of H<sub>2</sub>S on NOS activity has been investigated with mixed results and as appears common for the emerging area of H<sub>2</sub>S biology, the mechanisms for these observations have not been fully investigated. For example Kubo *et al.* [107, 108] showed recombinant NOS1 (rat), NOS2 (mouse) and to a latter extent, NOS3 (bovine) were inhibited by NaHS perhaps through interfering with tetrahydrobiopterin binding. Work by others [63, 109–111] subsequently showed that H<sub>2</sub>S inhibited NOS3 but not NOS2 activity in isolated rat and mouse aortic rings [107] and in human umbilical vein endothelial (HUVE) cells [109]. However, the lack of effect of NOS2 is not surprising since NOS2 is an inducible protein and was not induced in these experiments. Nevertheless, further experiments showed that intraperitoneal injection of NaHS into rats significantly reduced plasma NO<sub>2</sub><sup>−</sup>/NO<sub>3</sub><sup>−</sup> levels and suggested inhibition of NOS3 activity [109]. However, in each of these studies, free nitric oxide, H<sub>2</sub>S or S-nitrosothiols were not measured. It is therefore possible that the lowered NO<sub>2</sub><sup>−</sup>/NO<sub>3</sub><sup>−</sup> levels in plasma, as an index of nitric oxide, reflected the consumption of nitric oxide by either the added H<sub>2</sub>S gas, the added H<sub>2</sub>S donor (NaHS) or endogenously produced H<sub>2</sub>S *via* L-cysteine/pyridoxal phosphate in these studies.

It is also possible that H<sub>2</sub>S exerts its effects on nitric oxide synthesis *via* modulation of NOS substrate availability. For example in a rat model of pulmonary hypertension, induced by abdominal aorta-inferior cava vein shunt, L-arginine up-regulated CSE expression and H<sub>2</sub>S synthesis in pulmonary artery smooth muscle cells [111, 112]. However, in rat aortic tissue and HUVE cells H<sub>2</sub>S gas or H<sub>2</sub>S generated *via* L-cysteine/pyridoxal decreased L-arginine uptake and NO<sub>2</sub><sup>−</sup>/NO<sub>3</sub><sup>−</sup> formation through an apparently K<sub>ATP</sub> sensitive pathway [109], suggesting H<sub>2</sub>S could serve as a negative regulator of nitric oxide synthesis.

The effects of nitric oxide donors on H<sub>2</sub>S synthesis have also been investigated with species and tissue-dependent effects and contradictory findings reported. For example, the nitric oxide donor, sodium nitroprusside (SNP) up-regulates H<sub>2</sub>S production in rat vascular tissues and brain by increasing the expression of



**Table 3** Evidence consistent with nitric oxide–H<sub>2</sub>S cross-talk in the cardiovascular system

Cardiovascular observation
<b>Involvement in common cardiovascular pathology</b>
Endogenous H <sub>2</sub> S and nitric oxide levels negatively correlate with blood pressure and cardiac function
Haemorrhagic and endotoxemic shock induce elevated H <sub>2</sub> S and nitric oxide synthesis; prophylactic and therapeutic pharmacological inhibition of CSE or NOS2 is protective
Inflammation and oedema are associated with elevated H <sub>2</sub> S and nitric oxide levels; inhibition of CSE or NOS decreases inflammation and swelling
<b>Stimulatory effects of H<sub>2</sub>S on nitric oxide synthesis and nitric oxide mediated vasorelaxation</b>
The nitric oxide donor sodium nitroprusside (SNP) enhanced CBS activity and H <sub>2</sub> S levels <i>in vitro</i> and increased H <sub>2</sub> S synthesis in rat aortic, liver and ileum [63, 112]
The vasorelaxant effect of the nitric oxide donors SNP and SIN-1 were enhanced by incubating rat aortic tissue with NaHS [64]
L-NAME inhibited H <sub>2</sub> S-mediated vasorelaxation in rat aorta and the conversion of L-cysteine to H <sub>2</sub> S in rat aortic tissue. This effect was enhanced by treatment with the nitric oxide donor SNP [73]
NaHS [64, 65] or slow release H <sub>2</sub> S donor GYY4137 [79] prevented and reversed L-NAME-mediated hypertension in rats
H <sub>2</sub> S potentiated expression of NOS2 following stimulation of cultured rat vascular muscle cells with interleukin (IL)-1 $\beta$
H <sub>2</sub> S inhibited nitric oxide generation in isolated aortic tissues and <i>in vivo</i> [109] H <sub>2</sub> S down-regulated NOS3 (eNOS) but not NOS2 (iNOS) expression however, NOS2 was not induced [107, 108]
H <sub>2</sub> S, NaHS inhibited L-arginine uptake in human umbilical vein endothelial (HUVE) cells [109, 110]
L-cysteine / pyridoxal phosphate inhibited L-arginine uptake in HUVE cells; inhibited by PAG [109, 110]
L-Arginine increased CSE mRNA expression in pulmonary vascular endothelial and smooth muscle cells [110]
Nitric oxide donors up-regulated the expression and activity of CSE in vascular tissues and cultured aortic smooth muscle cells [74, 124]
Synergistic effect of nitric oxide and H <sub>2</sub> S on stonustoxin-induced relaxation of isolated rat aorta [123]
Synergistic effect of nitric oxide and H <sub>2</sub> S on rat pulmonary artery relaxation [64]
H <sub>2</sub> S-mediated ischemic after conditioning involved activation of NOS3 [121]
<b>Inhibitory effects of H<sub>2</sub>S on nitric oxide synthesis and nitric oxide mediated vasorelaxation</b>
NaHS inhibited recombinant NOS1 (nNOS) and NOS3 (eNOS) activity through interaction with BH <sub>4</sub> and NOS2 through unknown mechanisms [107–109]
L-NAME inhibited CSE expression and H <sub>2</sub> S synthesis in thoracic artery and superior mesenteric artery in rats [80]
Nitric oxide dependent relaxation of rat aortic rings through either nitric oxide donors (SNP, SNAP or SIN-1) or nitric oxide dependent (acetylcholine, histamine) but not nitric oxide independent ( <i>i.e.</i> isoprenaline) mediators was inhibited by exogenous (H <sub>2</sub> S gas, NaHS) and endogenous (L-cysteine/pyridoxal phosphate) H <sub>2</sub> S; an effect reversed by PAG [65]
L-NAME inhibited H <sub>2</sub> S-induced increase in mean arterial blood pressure in anaesthetized rats [65]
H <sub>2</sub> S inhibited vasorelaxation of isolated human internal mammary artery; induced by nitric oxide dependent mechanisms ( <i>i.e.</i> acetylcholine) [57]
PAG inhibited SNP-mediated vasorelaxation of isolated rat aorta [114]
<b>Other evidence of RNS-H<sub>2</sub>S interaction</b>
Scavenging of ONOO <sup>−</sup> ; inhibition of ONOO <sup>−</sup> -mediated cell death, intracellular protein nitration and tyrosine nitration <i>in vitro</i> [11]
Inhibition of tyrosine nitration <i>in vivo</i> in animal model of myocardial ischemia reperfusion [99]
Several nitric oxide donors react with NaHS <i>in vitro</i> to form a species resembling an inert 'nitrosothiol' [11, 26, 57, 65]
H <sub>2</sub> S-dependent nitrosothiol formation during lipopolysaccharide-induced septic shock in the rat [26]
NaHS-mediated decrease of O <sub>2</sub> <sup>−</sup> formation in human vascular smooth muscle cells was inhibited by the nitric oxide donor spermine NONOate [77]

CSE or CBS [110–112] suggesting a possible interaction of these gases at the expression of their synthesizing enzymes. In rat aortic tissue, the vasorelaxant effect of SNP was enhanced in the presence of NaHS. At the level of vascular smooth muscle cells, H<sub>2</sub>S has been reported to either enhance [63] or to attenuate [65, 75] the relaxant effect of nitric oxide in the rat aorta and human internal mammary artery [57]. Furthermore, NaHS-mediated inhibition of O<sub>2</sub><sup>•-</sup> production in HVSM was attenuated by nitric oxide (supplied as spermine NONOate) [64]. However, it is also possible that O<sub>2</sub><sup>•-</sup> was consumed by nitric oxide (forming ONOO<sup>-</sup>) or 'scavenged' by H<sub>2</sub>S [23–26].

## Evidence for the formation of a novel intermediate between nitric oxide and H<sub>2</sub>S at physiological pH

Given the reportedly high concentrations of H<sub>2</sub>S in the vasculature (Table 1) and the extensive tyrosine nitration (as a bio-marker for ONOO<sup>-</sup>) and NO<sub>2</sub><sup>-</sup>/NO<sub>3</sub><sup>-</sup> (as bio-markers for nitric oxide) observed in human tissues and fluids, (especially during pathology [4–7]), the reaction between H<sub>2</sub>S and ONOO<sup>-</sup> is plausible. Indeed, H<sub>2</sub>S was recently shown to react with ONOO<sup>-</sup> (or species derived from it at physiological pH) to inhibit nitrosative stress, cellular 3-nitrotyrosine formation and accompanying cell death [11]. However, since the half life of ONOO<sup>-</sup> at pH 7.4 and 37°C is <1 sec. [4–6], a much more plausible reaction is between nitric oxide (or intermediates derived from it) with H<sub>2</sub>S, especially since plasma levels of NO<sub>2</sub><sup>-</sup> (indicative of nitric oxide synthesis) are reported to reach high concentrations during chronic inflammation, oedema and sepsis; the precise conditions in which H<sub>2</sub>S synthesis is stimulated. Indeed, spectrophotometric, amperometric and electron paramagnetic resonance spectroscopy have recently shown that H<sub>2</sub>S (or intermediates derived from it at physiological pH) also reacts with nitric oxide (or derived species at physiological pH) to form a novel nitrosothiol-like compound(s) [26]. Further, incubation of RAW264.7 cells with nitric oxide donors (but not NaHS) resulted in cGMP accumulation but in sharp contrast, pre-mixing the nitric oxide donor SNP with NaHS did not result in cGMP formation unless Cu<sup>2+</sup> was added to release nitric oxide. This finding suggests that the 'nitrosothiol' did not increase cGMP levels itself but only after release of nitric oxide [26] and that this 'nitrosothiol' molecule was a relatively inert under these conditions. It is therefore tempting to speculate that the 'nitrosothiol'-like molecule may act as a sink for regulating nitric oxide and/or H<sub>2</sub>S bioavailability.

The H<sub>2</sub>S–nitric oxide interaction has also been investigated *ex vivo* and *in vivo* using exogenous and endogenously produced H<sub>2</sub>S and nitric oxide. In rat aortic ring preparations the addition several nitric oxide donors (SNP, SIN-1, SNAP) to phenylephrine pre-contracted rat aortic rings resulted in substantial relaxation but the subsequent addition of NaHS or authentic H<sub>2</sub>S gas

resulted in marked inhibition or reversal of nitric oxide donor-mediated relaxation [65]. However, pre-mixing nitric oxide donors with NaHS did not result in aortic ring relaxation unless Cu<sup>2+</sup> was added to release nitric oxide from H<sub>2</sub>S–nitric oxide [52] again suggesting that the 'nitrosothiol'-like molecule formed through the interaction of nitric oxide with H<sub>2</sub>S at physiological pH was inert. In addition, in isolated rat aortic rings [65] and human internal mammary artery [57], nitric oxide dependent (*i.e.* using histamine or acetylcholine) but not nitric oxide independent relaxation (*i.e.* using isoprenaline) was substantially inhibited by low concentrations of NaHS or H<sub>2</sub>S gas and this effect removed or reversed by the addition of Cu<sup>2+</sup> [65]. Nitric oxide dependent relaxation was also inhibited by the addition of L-cysteine/pyridoxal phosphate (as CSE substrate/co-factor), an effect reversed by the CSE inhibitor PAG [65]. Furthermore, we also showed that in liver homogenates from LPS-treated rats, 'nitrosothiol' formation was enhanced after the addition of L-cysteine/pyridoxal phosphate but inhibited by inclusion of PAG in the incubate [26]. These results suggest that H<sub>2</sub>S and nitric oxide or species derived from them at physiological pH, reacted together to form a molecule (presumably a nitrosothiol) which exhibited little or no vasorelaxant activity either *in vitro* or *in vivo*. Unfortunately, at present the precise identity, molecular structure and physiological properties of this nitrosothiol-like species are currently unknown.

## Concluding remarks

The precise role of H<sub>2</sub>S under physiological and/or pathophysiological conditions remains unclear. The molecular interaction between NOS–CSE, nitric oxide–CSE, H<sub>2</sub>S–NOS and nitric oxide–H<sub>2</sub>S are ill-defined and require further attention. However, from the studies outlined above, it is likely that the physiological role of H<sub>2</sub>S might be to suppress the vasodilator effect of nitric oxide through two distinct mechanisms; inhibition of NOS3 and 'scavenging' of nitric oxide resulting in the enhancement of vascular contractility. Conversely, under pathophysiological conditions whereby nitric oxide and H<sub>2</sub>S synthesis are greatly increased (*i.e.* sepsis and shock) the vasculoprotective action of H<sub>2</sub>S and H<sub>2</sub>S-mediated nitric oxide 'quenching' are lost resulting in excessive and pathological loss of vascular tone. The isolation and molecular identification of the nitrosothiol-like species derived from H<sub>2</sub>S and nitric oxide will greatly facilitate the unravelling of the complex interaction between these gases and could address the varied and contradictory reports on the physiology and pathophysiology of H<sub>2</sub>S and nitric oxide.

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